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RELATIONSHIP BETWEEN LIGHT INDUCED EPR SIGNAL AND PIGMENT P700

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In two previous communications (1,2) we have reported observations on the narrow (~8 gauss) EPR signal induced by light in photosynthetic materials. (This signal is also known as signal I (Ref. 3) or signal II (Ref. 4) and rapidly decaying or "R" signal (Ref. 5). The first paper (1) described properties of the signal observed in a preparation of the red alga strain TX 27 that was largely deprived of phycobilin and enriched in "P700" by partial extraction of the chlorophyll using 72% acetone (6). The signal appeared on illumination at room or liquid nitrogen temperature or after chemical oxidation by means of ferricyanide. Double integration of the derivative signal indicated a concentration of spins nearly identical to the concentration of P700 as determined by difference spectroscopy assuming a molar extinction of 10^{-5} $M^{-1}xcm^{-1}$ and complete bleaching in the photoact. A second extraction of the algal preparation, now with 80% instead of 72% acetone, removed the EPR signal as well as the difference spectroscopic signal at 700 mu. We concluded that these results could be most readily explained if the photooxidized form of P700 was responsible for the narrow, fast decaying EPR signal in photosynthesis.

The second paper (2) reported observations with whole cells of the blue-green alga Anacystis at room temperature. The relative strength of the narrow EPR signal was measured as a function of intensity of either one or both of two wavelengths: 630 mu absorbed by phycocyanin and quite effective in provoking photosynthesis and 710 mu absorbed by (long wave) chlorophyll and rather ineffective in provoking photosynthesis. The EPR signal showed the same behavior as the oxidized form of P700 which was observed earlier spectroscopically (7): Long wave light proved much more effective in provoking the EPR signal than short wavelengths, or a combination of the two lights. These data therefore also indicated the possible identity of the EPR signal with oxidized P700, presumably the oxidized moiety generated in the long wave photoreaction of photosynthesis. Since its reduction requires the reduced moiety generated in the short wave photoact, $P700^{+}$ tends to accumulate in the absence of the short wave light.

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Weaver and Bishop (8) recently failed to observe the narrow EPR signal in a Scenedesmus mutant (#8). Spectroscopic examination of this alga indeed did not reveal a light induced bleaching of P700 (9).

Spectroscopic determination of P700 revealed a maximum absorbancy change at 700 mu of 1 unit per 300-400 units of total chlorophyll absorbancy (at ~675 mu). Its soret band (at 432 mu) as well as its nearly identical solubility in organic solvents indicate P700 is a chlorophyll a molecule. We assume that a special binding site causes a long wave change of its absorption band and underlies its function as a photoconverter. This assumption assigns to P700 a molar extinction coefficient similar to that of chlorophyll a ($\sim 10^5$ in vivo).

A second assumption: that complete bleaching occurs in the photoact yields a ratio of one trapping center per 300-400 sensitizing chlorophyll molecules--in good agreement with measurements of the "photosynthetic unit" (10,11).

If the oxidized form of P700, $P700^+$, which is formed in the long wavelength photoact, were identical with the free radical species observed by EPR, the quantitative relationship, between the number of spins represented by the EPR signal and the amount of P700 detected optically, which we observed in a preliminary experiment (1), should hold rather generally for photosynthetic materials and furthermore, the kinetics of appearance and disappearance of the EPR signal should match that of the typical absorption band of P700.

In the present work we undertook a direct approach at quantitation of the number of spins represented by the light induced EPR signal and the amount of chlorophyll and P700 present in a variety of materials. Knowledge of any consistent quantitative relationships would obviously be of interest, even if the EPR signal were not due to $P700^+$ itself or a closely associated electron carrier or trap.

We were aware during the course of this work that quantitation of EPR signals is beset with many difficulties, that we had to make certain assumptions, which are not readily amenable to experimental verification, and that a definitive identification of the component responsible for the EPR signal could not be expected from our approach. The EPR data were evaluated on the assumption that the light induced free

radical species are fully detectable by EPR and that no interactions interfere with this detectability.

The concentration of unpaired electrons was measured by EPR spectroscopy at ambient and low (-50° to -70°) temperature as will be described in detail elsewhere (12). For the experiments at room temperature a double cavity was used, which held both sample and standard in matched flat cells. A benzene solution of diphenylpicrylhydrazyl (DPPH) was used as a standard at room temperature and a pitch sample in KCl and nitrosyldisulfonate in KOH at low temperature. The standards as well as the integration procedure used were cross checked. Conditions were chosen that saturation of the EPR signals with microwave power did not occur or was sufficiently small that it could be corrected. In order to ensure saturation of the pigment suspension with light, i.e. maximal signal development, the signal amplitude of serial dilutions of these suspensions was measured until a linear relationship between signal amplitude and concentration was observed. The double integration of the derivative signals, which resulted in the quantitative estimate of unpaired spins, was based on signals obtained in this linear region. Values were also observed in the dark and after addition of ferricyanide. The value obtained with ferricyanide can in many cases serve as a control for light saturation and maximal signal development, as the concentration of unpaired electrons produced by an excess of this oxidant is either very similar to that obtained on illumination under saturating conditions or larger. Only materials were selected for study in which overlapping signals (broad signal, 20 gauss, slow decaying or "S" signal, and Mn (II) signal) were absent or small. Overlap, when occurring, was corrected.

Results obtained under satisfactory experimental conditions are summarized in the table. The last vertical column gives the calculated ratio of unpaired spins per P700. Most values cluster around a ratio of 2, although the low temperature experiments on the TX 27 preparations and the experiments on Anacystis yielded higher ratios. We have no explanation for the high values obtained at low temperature. Although a different standard (pitch) was used at low temperature than at room temperature, a comparison of both standards gave excellent agreement. It is likely that the high values obtained for whole Anacystis and the same preparation after sound treatment are due to overlap of the narrow EPR signal with the broad light induced EPR signal in such

complex preparations. Although overlap was corrected on the basis of the dark signals observed after illumination, this correction is not entirely satisfactory as the broad signal is more intense during illumination. In the last two lines determinations on chromatophores from Rhodospirillum rubrum are reported. Since P700 is not a constituent of these organisms only the ratio of bacterio chlorophyll [determined according to (13)] to unpaired spins is given. Approximately 3% of this chlorophyll are thought to represent a photoconverter, P890, similar to P700. On this basis a spin per P890 ratio of 0.4 to 0.5 would be obtained.

We are aware that our experiments cannot provide a final decision or an identification of the narrow light induced EPR signal; they could at best rule out or make appear plausible certain possible interpretations. In assessing the significance of the values we obtained, two principal considerations are pertinent: The first is conserned with the accuracy of our quantitation procedures and the second with the question of whether all radicals and radical species formed in the illuminated samples were, in fact, detected by EPR.

To the first point we can say that use of the double sample cavity and carefully matched cells, the use of independently standardized standards, attention to the conditions of saturation with light and microwave power and the consistency of the results obtained in the determinations at both room and low temperature, make it very unlikely that gross errors were committed. Nevertheless, in view of the uncertainties in the absolute values of EPR standards and in comparison of different materials, we think that accumulation of errors could have led to values which are in error by a factor of 2 or 3. The consistancy of the results indicates that these errors, if incurred, are not random but systematic and due to certain incorrect assumptions.

The second point of concern is related to the question as to what type of paramagnetic species is in fact responsible for the observed signal. The simplest assumption, on which our experiments here are based, is that a single free radical species arises, which has a structure and environment such that it can be quantitatively detected by the EPR technique. However, since a one electron oxidation produces the radical, it appears possible that a second radical, formed by the corresponding one electron reduction (in the extreme case a free electron) is simultaneously generated. There are no

indications we know of from the behavior of the observed EPR signal that it may represent two different species. This would still not exclude such a possibility, for which one could see some support in the high ratios of free spins produced per molecule of P700 (cf. Table 1).

A serious objection to the interpretation of our experimental results could arise from the possibility that we might not be detecting more than a fraction of the radicals actually produced. Several explanations could be given for this. We may be dealing with "lifetime" broadening, i.e., short relaxation time of the unpaired electron; an exchange interaction may broaden the line; or, in case a free electron were generated, it could be trapped at non-equivalent sites and therefore, experience varying local magnetic influences, which would lead to line broadening. Such arguments cannot at present, be refuted on experimental grounds. If they were valid our values would set the lower limit of radical concentration.

We may then conclude from our data in the light of these considerations that the number of unpaired electrons induced by light in the photosynthetic material studied is either closely similar or bigger, certainly not smaller than the amount of P700 present. It is of interest to note that other components of the photosynthetic system in plants, have been reported to occur at a concentration of the same range as P700 and the light induced EPR signal studied here. However, the metal constituents of two of these: cytochrome f and plastocyanin are certainly not responsible for this signal.

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Table 1

Concentrations of Chlorophyll, P700 and Detectable Light-Induced Free Radicals in Various Photosynthetic Materials

Material	Temp. °C	Chlorophyll 10^{-5}M	P_{700} 10^{-7}M	Spins 10^{-7}M	Ratio: Chlorophyll per spins	Ratio: Spins per P_{700}
TX 27	25	72	38	67	108	1.8
broken, washed	-53	2.5	1.3	4.3	58	3.3
TX 27, washed	25	50	65	110	45	1.7
acetone extracted	-53	2.0	2.6	9.0	22	3.5
Anacystis	25	120	40	107	112	2.7
whole cells	-53	6	2	11	55	5.5
Anacystis	25	52	17	65	80	3.8
broken, washed	-53	2.7	0.9	3.8	71	4.2
Chloroplasts						
acetone extracted	-72	24	35	79	30	2.3
Chloroplasts						
fresh	-70	100	25	51	196	2.0
aged	-70	72	18	38	190	2.1
acetone extracted	-70	16	23	58	28	2.6
R.rubrum	25	210		330	64	
	-53	5.3		6.3	84	

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